

(g) the amino acid sequence from the 149th to 238th position of green fluorescent protein.--

BASIS FOR THE AMENDMENT

Claims 29 and 33 have been amended.

All amendments to the specification and claims are supported by the application as originally filed, and serve to provide proper reference to the appropriate sequence identifiers.

No new matter is believed to be introduced by virtue of the present amendment.

REMARKS

Claims 1 - 64 are pending in the present application.

Applicants have now submitted a substitute Sequence Listing and a corresponding computer-readable Sequence Listing. The sequence information recorded in the corresponding computer-readable Sequence Listing is identical to the paper copy of the substitute Sequence Listing. Support for all of the sequences listed in the substitute Sequence Listing is found in the present application as originally filed. No new matter is believed to have been introduced by the submission of the substitute Sequence Listing and the corresponding computer-readable Sequence Listing.

Applicants submit that the present application is ready for examination on the merits.

Early notice to this effect is earnestly solicited.

Respectfully submitted,

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IN THE SPECIFICATION

Please replace the paragraph at page 10, prenumbered line 18, to page 11, prenumbered line 6, as follows:

--[14] A biosensor protein comprising the following sequences (a) to (h) sequentially from the N terminus:

- (a) Met-Gly-Thr or Met-Val-Asp (linker X);
- (b) a partial amino acid sequence of myosin light chain kinase protein (Ser-Ser-Arg-Arg-Lys-Trp-Asn-Lys-Thr-Gly-His-Ala-Val-Arg-Ala-Ile-Gly-Arg-Leu-Ser-Ser) [SEQ ID NO:6]
- (c) Leu-Glu (linker Y);
- (d) the amino acid sequence from the 149th to 238th position of green fluorescent protein;
- (e) Gly-Gly-Thr-Gly-Gly-Ser (linker amino acid sequence) [amino acid 117 to 122 of SEQ ID NO:8]
- (f) the amino acid sequence from the 1st to 144th position of green fluorescent protein;
- (g) Gly-Thr-Arg or Thr-Arg (linker Z); and
- (h) the amino acid sequence from the 2nd to 148th position of rat calmodulin protein.--

Please replace the paragraph at page 12, prenumbered line 14, to page 13, prenumbered line 1, as follows:

--[18] A biosensor protein comprising the following sequences (a) to (g) sequentially from the N terminus:

- (a) the amino acid sequence from the 1st to 144th position of green fluorescent protein;
- (b) Gly-Thr-Arg (linker A);
- (c) the amino acid sequence from the 2nd to 148th position of rat calmodulin protein;
- (d) Gly-Thr or Gly-Thr-Gly-Ser-Gly-Gly-Ser (linker B) [SEQ ID NO:17];
- (e) a partial amino acid sequence of myosin light chain kinase protein (Ser-Ser-Arg-Arg-Lys-Trp-Asn-Lys-Thr-Gly-His-Ala-Val-Arg-Ala-Ile-Gly-Arg-Leu-Ser-Ser) [SEQ ID NO:6];
- (f) Thr-Ser (linker C); and
- (g) the amino acid sequence from the 149th to 238th position of green fluorescent protein.--

Please replace the paragraph at page 19, prenumbered lines 6-18, as follows:

--In the present invention, the modified GFP preferably means those constructed by the steps below:

cleaving original GFP in the vicinity of the hotspot amino acid residue (e.g., between the n-th amino acid and the (n+l)th amino acid of GFP);

removing the amino acids in the cleavage site (e.g., amino acids from the (n-a)th to the n-th position of GFP), thereby modifying the molecular structure of GFP; and

linking the N terminus and the C terminus of original GFP with appropriate linker amino acids (e.g., Gly-Gly-Thr-Gly-Ser [amino acids 117 to 122 of SEQ ID NO:8]) in case of necessity (see FIG. 1C).--

Please replace the paragraph at page 19, prenumbered line 17, to page 20, prenumbered line 7, as follows:

--The linker amino acids for connecting the N terminus and C terminus of original GFP preferably have an amino acid sequence consisting of 2 to 10 amino acid molecules. The linker amino acids more preferably have an amino acid sequence rich in glycine. Particularly preferable example of the linker amino acids includes Gly-Gly-Thr-Gly-Gly-Ser [amino acids 117 to 122 of SEQ ID NO:8]. However, the linker amino acid sequence is not restricted to this example.--

Please replace the paragraph at page 37, prenumbered lines 18-25, as follows:

--The linker of sequence (e) is preferably an amino acid sequence consisting of 2-10 amino acids. The linker of sequence (e) is more preferably an amino acid sequence rich in an amino acid (e.g., glycine) which allows a main chain of a peptide to move with a large degree of freedom, rendering the main chain flexible. Further preferably, the linker of sequence (e) is the sequence of Gly-Gly-Thr-Gly-Gly-Ser [amino acids 117 to 122 of SEQ ID NO:8].--

Please replace the paragraph at page 41, prenumbered lines 13-24, as follows:

--The amino acid sequence of rat calmodulin used herein as a functional molecule is represented by Sequence ID No:[2]4. Each amino acid from the 1st to 148th position of the amino acid sequence represented by Sequence ID No:[2]4 corresponds to the "n-th amino acid of calmodulin protein" described in this text. In the amino acid sequence represented by Sequence ID No:[2]4, methionine of the N terminus is omitted therefrom. This is the reason why methionine of the N terminus is cleaved out by post-translational modification, after translation of genetic code of calmodulin into amino acids.--

Please replace the paragraph at page 42, prenumbered lines 5-23, as follows:

--In Tables 1 and 2, the names of probe No. are given to a variety of biosensor proteins listed therein. For example, the one-dimensional structure of the probe No. G3 (hereinafter, also referred to as "G3") is shown in FIG. 2. The G3 has, in this order from the N terminus,

linker X (Met-Gly-Thr);

a partial amino acid sequence of myosin light chain kinase (Ser-Ser-Arg-Arg-Lys-Trp-Asn-Lys-Thr-Gly-His-Ala-Val-Arg-Ala-Ile-Gly-Arg-Leu-Ser-Ser) [SEQ ID NO:6];

linker Y (Thr-Ser);

the modified GFP (the amino acid sequence of the 149-238th position of GFP, the linker amino acids (Gly-Gly-Thr-Gly-Gly-Ser) [amino acids 117 to 122 of SEQ ID NO:8], and the amino acid sequence of the 1-144th position of GFP, in this order from the N terminus);

linker Z (Gly-Thr-Arg); and

the amino acid sequence of the 2nd to 148th position of rat calmodulin---

Please replace the paragraph at page 42, prenumbered line 24, to page 44, prenumbered line 3, as follows:

--In Tables 1 and 2, the amino acid sequences in each column of Linkers X, Y and Z are represented by single-letter notation. The symbol "O" in the column of M13 refers to the sequence of (Ser-Ser-Arg-Arg-Lys-Trp-Asn-Lys-Thr-Gly-His-Ala-Val-Arg-Ala-Ile-Gly-Arg-Leu-Ser-Ser) [SEQ ID NO:8]. The number "149-144" in the column of cpEGFP means that the modified GFP has, in this order from the N terminus, the amino acid sequence of the 149-238th position of GFP, the linker amino acids (Gly-Gly-Thr-Gly-Gly-Ser) [amino acids 117 to 122 of SEQ ID NO:8], and the amino acid sequence of the 1-144th position of GFP. The symbol "O" in the column of CaM represents the amino acid sequence of the 2nd to 148th

position of original rat calmodulin, and the symbol "CN" represents the amino acid sequence of the 2nd to 148th position of calmodulin mutant CaMCN (Persechini A et al., 1997, Cell Calcium 22, 209-216). The symbol "+" in the column of Fluorescence means that a biosensor protein emits weak fluorescence before ATP is added, whereas the symbol "-" means that a biosensor protein emits no fluorescence before ATP is added. Further, the symbol "*" represents that a biosensor protein emits weak fluorescence at a high intracellular calcium concentration of about 2 mM. The value " $\Delta F/F$ " in the column of ATP response is a value (arbitrary unit) obtained by dividing " ΔF " by "F", wherein " ΔF " is a change amount of fluorescence intensity between before and after addition of ATP and "F" is a fluorescence intensity before addition of ATP, in the cell (HEK cell) producing a biosensor protein. The symbol "nt" represents "not tested". The symbol "+" in the column of Photoisomerization means that a biosensor protein causes photoisomerization, and the symbol "-" means that a biosensor protein causes no photoisomerization.--

Please replace the paragraph at page 50, prenumbered line 13, to page 51, prenumbered line 4, as follows:

--In Tables 3 and 4, the names of probe No. are given to various biosensor proteins listed therein. For example, the one-dimensional structure of the probe No. G85 (hereinafter, also referred to as "G85") is shown in FIG. 2. The G85 has, in this order from the N terminus,

linker X (Met-Val-Asp);

a partial amino acid sequence of myosin light chain kinase (Ser-Ser-Arg-Arg-Lys-Trp-Asn-Lys-Thr-Gly-His-Ala-Val-Arg-Ala-Ile-Gly-Arg-Leu-Ser-Ser) [SEQ ID NO:6];

linker Y (Leu-Glu);

the modified GFP (the amino acid sequence of the 149-238th position of GFP, the linker amino acids (Gly-Gly-Thr-Gly-Gly-Ser) [amino acids 117 to 122 of SEQ ID NO:8], and the amino acid sequence of the 1-144th position of GFP, in this order from the N terminus);

linker Z (Thr-Arg); and

the amino acid sequence of the 2nd to 148th position of rat calmodulin---

Please replace the paragraph at page 51, prenumbered line 5, to page 52, prenumbered line 2, as follows:

--In Tables 3 and 4, the amino acid sequences of the linkers X, Y and Z are represented by single-letter notation. The symbol "O" in the column of M13 represents the sequence of (Ser-Ser-Arg-Arg-Lys-Trp-Asn-Lys-Thr-Gly-His-Ala-Val-Arg-Ala-Ile-Gly-Arg-Leu-Ser-Ser) [SEQ ID NO:6]. The number "149-144" in the column of cpEGFP means that the modified GFP has, in this order from the N terminus, the amino acid sequence from the 149-238th position of GFP, the linker amino acids (Gly-Gly-Thr-Gly-Gly-Ser) [amino acids 117 to 122 of SEQ ID NO:8], and the amino acid sequence of the 1-144th position of GFP. The symbol "O" in the column of CaM represents the amino acid sequence of the 2nd to 148th position of rat-inherent calmodulin. The value " $\Delta F/F$ " in the column of ATP response is a value (arbitrary unit) obtained by dividing " ΔF " by "F", wherein ΔF is a change amount of fluorescence intensity between before and after addition of ATP and "F" is a fluorescence intensity before addition of ATP, in the cell (HEK cell) producing a biosensor protein. The column "n" refers to the number of cells tested. The symbol "+" in the column of Photoisomerization means that a biosensor protein causes photoisomerization, whereas the symbol "-" means that a biosensor protein causes no photoisomerization.--

Please replace the paragraph at page 57, prenumbered line 12, to page 58, prenumbered line 1, as follows:

--Particularly preferable example of the calcium sensor protein of the present invention is characterized by comprising the following sequences (a) to (h), sequentially from the N terminus,

- (a) Met-Gly-Thr or Met-Val-Asp (linker X);
- (b) a partial amino acid sequence of myosin light chain kinase protein (Ser-Ser-Arg-Arg-Lys-Trp-Asn-Lys-Thr-Gly-His-Ala-Val-Arg-Ala-Ile-Gly-Arg-Leu-Ser-Ser) [SEQ ID NO:6];
- (c) Leu-Glu (linker Y);
- (d) the amino acid sequence of the 149th to 238th position of green fluorescent protein;
- (e) Gly-Gly-Thr-Gly-Ser [amino acids 117 to 122 of SEQ ID NO:8];
- (f) the amino acid sequence of the 1st to 144th position of green fluorescent protein;
- (g) Gly-Thr-Arg or Thr-Arg (linker Z); and
- (h) the amino acid sequence from the 2nd to 148th position of rat calmodulin protein.--

Please replace the paragraph at page 60, prenumbered lines 11-18, as follows:

--The calcium sensor of probe No. G85 is made by expressing the gene of the calcium sensor in the HEK 293 cells, and then a factor known to increase the calcium ion concentration in cells, such as ATP (0.1 mM) or carbachol (CCH) (0.1 mM), is added to the HEK 293 cells. The reaction profile of the calcium sensor G85 is shown in FIG. 3. The nucleotide sequence of the G85 is represented by Sequence ID No:[4]7.--

Please replace the paragraph at page 62, prenumbered lines 9-21, as follows:

--The fragment M13 (Sequence ID No:[3]6) of smooth muscle myosin light chain kinase was prepared using the following synthetic primers which have been synthesized by Katayama Chemical. More specifically, PCR was performed using the following two primers as templates and primers, thereby synthesizing the fragment M13 of smooth muscle myosin light chain kinase.

smMLCKM13-1 primer:

GCGCTAGCCGCCACCATGGGTACCTCATCACGTCGAAGTGGAATAAGAC
AGGTCACGCAGTCAGA (Sequence ID No: [5]9)

smMLCKM13-2 primer:

GGCGCGGCCGCTCAACTAGTTGAGCTAGCCGACCTATAGCTCTGACTGC
GTGACCTGTCTT (Sequence ID No: [6]10)--

Please replace the paragraph at page 63, prenumbered lines 4-9, as follows:

--EGFP-31 primer:

GGACGCGTACTAGTAACGTCTATATCATGGCCGAC (Sequence ID No: [7]11)

EGFP-20 primer:

CCGGTACCGCCCTTGTACAGCTCGTCCATGCC (Sequence ID No: [8]12)--

Please replace the paragraph at page 63, prenumbered lines 16-21, as follows:

--EGFP-21 primer:

CCGGTACCGGAGGGAGCATGGTGAGCAAGGGCGAGGAG (Sequence ID No:
[9]13)

EGFP-30 primer:

GGACGCGTCCCCTTGTACTCCAGCTTGTGCC (Sequence ID No: [10]14--

Please replace the paragraph at page 63, prenumbered line 27, to page 64,
prenumbered line 4, as follows:

--PCR was performed using the following primers to rat calmodulin and a plasmid rCaM encoding a rat calmodulin cDNA (Sequence ID No: [2]3; Accession No. M19312 (Mori M, 2000, Biochemistry 39, 1316-1323)) as a template.--

Please replace the paragraph at page 64, prenumbered lines 5-8, as follows:

--rCaM-2 primer: GGACGCGTGACCAACTGACTGAAGAGCAG

(Sequence ID No: [11]15)

rCaM-10 primer: GCGCGGCCGCTCACTTCGCTGTCATCATTGTAC

(Sequence ID No: [12]16--

Please delete the original sequence listing.

Page 83 (Abstract), after the last line, beginning on a new page, please insert the attached substitute sequence listing.

IN THE CLAIMS

Please amend the claims as follows:

--29. (Amended) A biosensor protein comprising the following sequences (a) to (h) sequentially from the N terminus:

- (a) Met-Gly-Thr or Met-Val-Asp (linker X);
- (b) a partial amino acid sequence of myosin light chain kinase protein Ser-Ser-Arg-Arg-Lys-Trp-Asn-Lys-Thr-Gly-His-Ala-Val-Arg-Ala-Ile-Gly-Arg-Leu-Ser-Ser) [SEQ ID NO:6];
- (c) Leu-Glu (linker Y);
- (d) the amino acid sequence from the 149th to 238th position of green fluorescent protein;

(e) Gly-Gly-Thr-Gly-Gly-Ser (linker amino acid sequence; amino acids 117 to 122 of SEQ ID NO:8);

(f) the amino acid sequence from the 1st to 144th position of green fluorescent protein;

(g) Gly-Thr-Arg or Thr-Arg (linker Z); and

(h) the amino acid sequence from the 2nd to 148th position of rat calmodulin protein.--

--33. (Amended) A biosensor protein comprising the following sequences (a) to (g) sequentially from the N terminus:

(a) the amino acid sequence from the 1st to 144th position of green fluorescent protein;

(b) Gly-Thr-Arg (linker A);

(c) the amino acid sequence from the 2nd to 148th position of rat calmodulin protein;

(d) Gly-Thr or [Gly-Thr-Gly-Ser-Gly-Gly-Ser] (linker B; SEQ ID NO:17);

(e) a partial amino acid sequence of myosin light chain kinase protein (Ser-Ser-Arg-Arg-Lys-Trp-Asn-Lys-Thr-Gly-His-Ala-Val-Arg-Ala-Ile-Gly-Arg-Leu-Ser-Ser) [SEQ ID NO:6];

(f) Thr-Ser (linker C); and

(g) the amino acid sequence from the 149th to 238th position of green fluorescent protein.--

